

## Rituximab: A Boot to Protect the Foot

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Given the shortage of organs available for transplantation and the rising prevalence of ESRD,<sup>1</sup> new avenues including xeno-kidney transplantation need to be explored. Despite tremendous advances in achieving immunologic hyporesponsiveness in xeno-kidney transplantation, long-term organ survival remains a major challenge.<sup>2</sup> Although dramatic improvements have recently been achieved by genetic deletion of a major xenoantigen causing hyperacute rejection in experimental donors ( $\alpha$ -1,3-galactosyltransferase),<sup>3</sup> most recipients still experience additional barriers limiting graft survival, such as acute vascular rejection and severe post-transplant proteinuria. In this issue of *JASN*, Tasaki *et al.* suggests that rituximab, an immunomodulator targeting CD20 on B lymphocytes, could delay onset of post-transplant proteinuria in baboons transplanted with kidneys from  $\alpha$ -1,3-galactosyltransferase-deficient pigs when the drug was administered in the peritransplant period.<sup>4</sup> This discovery marks another important step toward a potential use of xenografts in nephrology.

The possibility that rituximab reduces proteinuria has been reported on several occasions. Interestingly, the study of Tasaki *et al.* demonstrates that protection from proteinuria was not associated with B-lymphocyte depletion and required kidney graft expression of sphingomyelin phosphodiesterase acid-like 3b (SMPDL-3b). Rituximab was able to bind directly to CD20-negative glomerular epithelial cells and to immunoprecipitate the 50-kD isoform of SMPDL-3b from pig glomerular lysates. Rituximab prevented both pig podocyte injury and the suppression of SMPDL-3b expression caused by exposure to naive baboon sera. This was

associated with preservation of cell viability. Additional key novel experiments demonstrated that preformed natural antibodies are necessary in combination with complement for the baboon sera to reduce podocyte viability. In particular, exposure of cultured podocytes to untreated serum compromised podocyte viability only in the presence of complement, and this phenomenon was prevented when serum was pre-absorbed.

The protection of podocyte function in xenotransplantation by rituximab does not come as a total surprise. We have previously demonstrated that rituximab binds SMPDL-3b, a protein with a rituximab-binding amino acid sequence that is expressed in podocyte lipid rafts.<sup>5</sup> Rituximab protects podocytes through the preservation of SMPDL-3b and acid sphingomyelinase expression and activity from sera-induced injury when the sera of patients with FSGS are used.<sup>5</sup> However, the fraction of the sera responsible for down-regulation of SMPDL-3b was hitherto unknown; Tasaki *et al.* have now shown that preformed natural antibodies are indeed responsible for xenotransplant-related proteinuria. If, and how, antibodies also play a role in the downregulation of SMPDL-3b that we have observed in FSGS remains to be established.

Tasaki *et al.* demonstrated that peritransplant administration of rituximab delayed, but did not prevent, the post-transplant occurrence of proteinuria. The authors interpreted this observation as resulting from persistent preformed antibodies once the rituximab levels fell over time. In prior studies, when a single dose of rituximab was administered to patients with FSGS in the peritransplant period, 26% of these patients still experienced nephrotic-range proteinuria in the first 30 days after transplant,<sup>5</sup> suggesting that additional mechanisms may be involved or work in parallel. First, rituximab may be binding to SMPDL-3b on the surface of podocytes, which probably interferes with activation of podocyte  $\alpha$ V $\beta$ 3 integrin by circulating factors, such as the soluble urokinase plasminogen activator receptor.<sup>6,7</sup> The role of these circulating factors should also be explored in xenograft-related post-transplant proteinuria. Second, upregulation of additional molecules that may serve as danger signals in podocytes may occur, as the authors described preliminary upregulation of CD80 in their transplant model, similar to podocyte CD80 expression under disease conditions.<sup>8</sup> Third, unique to the transplant models used by Tasaki *et al.* is the fact that recipient baboons underwent splenectomy and thymectomy before transplant. Because a high incidence of anti-nephrin antibodies have been reported in experimental models of graft-versus-host disease<sup>9</sup> (which may cause nephrotic-range proteinuria<sup>10</sup>), and nephrin is expressed in the thymus (where it likely represents an antigen to induce tolerance<sup>11</sup>), it would be interesting to know

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whether xenotransplant recipients develop anti-nephrin antibodies, and whether rituximab modulates such phenomenon independently of its interaction with SMPDL-3b. The fact that, in Tasaki and colleagues' study, SMPDL-3b expression and not B-lymphocyte depletion strongly correlated with response to rituximab favors the first two options as the more likely to occur.

Tasaki and colleagues' findings confirm the potential beneficial off-target mechanisms of action of rituximab, which was initially developed to specifically target CD20 in B lymphocytes. These observations are a great example how off-target effects or adverse effects of commonly used drugs can allow for new target identification. Such an approach is likely to have a high probability of success for drug development because it is bolstered by clinical effects.

Several unanswered questions remain to be addressed. How can monoclonal antibodies administered as part of a prevention strategy reach podocytes? Is the 8% immunoglobulin component seen on electrophoresis of normal urine sufficient to provide therapeutic protection? Is SMPDL-3b a gate to danger signals in podocytes? Is this specific to certain danger signals or not? Is it possible that SMPDL-3b simply affects the distribution of lipids on caveolar pits and, therefore, alters the pattern of localization and the turnover of molecules at the plasma membrane? These are all very important aspects to study before we might consider SMPDL-3b as a new target for podocyte protection and drug development.

## DISCLOSURES

J.R. and A.F. have pending or issued patents on novel kidney protective drug therapies. They stand to gain royalties from their commercialization.

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See related article, "Rituximab Treatment Prevents the Early Development of Proteinuria following Pig-to-Baboon Xeno-Kidney Transplantation," on pages 737–744.

## Notch Ties a Knot on Fistula Maturation

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The arteriovenous fistula (AVF) is the preferred type of vascular access for maintenance hemodialysis because AVFs that mature successfully require fewer interventions, have lower rates of infection, and function longer than synthetic arteriovenous grafts or central venous catheters. However, the advantages of this vascular access type are less compelling if one considers that up to 60% of AVFs fail to mature sufficiently to provide adequate dialysis.<sup>1</sup>

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